

We claim:

34 Claim 1. A method for the treatment of a particular volume of plant or animal tissue, the method comprising the steps of:

(a) treating the plant or animal tissue with at least one photo-active agent, wherein the particular volume of the plant or animal tissue retains at least a portion of the at least one photo-active agent; and

(b) treating the particular volume of the plant or animal tissue with light to promote a multi-photon photoactivation of at least one of said at least one photo-active agent retained in the particular volume of the plant or animal tissue, wherein the at least one excited photo-active agent becomes photo-activated in the particular volume of the plant or animal tissue.

42 Claim 2. The method of Claim 1 wherein the light to promote said multi-photon photoactivation is a laser light produced by a laser.

43 Claim 3. The method of Claim 2 wherein the laser light comprises a train of one or more ultrashort pulses.

44 Claim 4. The method of Claim 3 wherein each of said one or more pulses has a duration of at most approximately 10 ps.

45 Claim 5. The method of Claim 2 including operating the laser to produce light at a wavelength between approximately 500 nm to 4000 nm.

44 Claim 6. The method of Claim 1 wherein said light is at a wavelength between approximately 500 nm to 4000 nm.

47 Claim 7. The method of Claim 1 wherein the light to promote said multi-photon photoactivation is a focused beam of light.

48 Claim 8. The method of Claim 7 wherein the focused beam of light is focused laser light.

49 Claim 9. The method of Claim 1 wherein said step of treating the particular volume of the plant or animal tissue includes positioning a focus of a beam of light over a range of positions so that a focal plane of the light beam occurs at a site located between a surface of the tissue and a point substantially beyond the tissue surface, whereby said step of 5 treating the particular volume of the plant or animal tissue may extend to penetrate deep within the tissue.

50 Claim 10. The method of Claim 9 further including varying, while the beam of light 54 is extant, the radial position of the focal plane within the tissue, thereby to photoactivate the at least one photo-active agent at a multiplicity of positions between the tissue surface and a position located substantially beyond the tissue surface.

Claim 11. The method of Claim 1 wherein said at least one photo-active agent becomes photoactivated in said particular volume at a controllable position substantially beyond a tissue surface.

51 Claim 12. The method of Claim 11 wherein said treating step includes directing a laser light to said particular volume.

52 Claim 13. The method of Claim 12 wherein said treating step includes directing a pulsed laser light to said particular volume.

53 Claim 14. The method of Claim 13 wherein said laser is pulsed to produce pulses of at most approximately 10 ps.

Claim 15. The method of Claim 1 wherein said multi-photon photoactivation includes an essentially simultaneous interaction of at least two photons with said agent so as to produce a photo-activated agent.

Claim 16. The method of Claim 1 wherein said agent is selected from the group comprising psoralen derivatives; porphyrin and hematoporphyrin derivatives; chlorin derivatives; phthalocyanine derivatives; rhodamine derivatives; coumarin derivatives; benzophenoxazine derivatives; chlorpromazine and chlorpromazine derivatives; chlorophyll and bacteriochlorophyll derivatives; pheophorbide a (Pheo a); merocyanine 540 (MC 540); Vitamin D; 5-amino-laevulinic acid (ALA); photosan; pheophorbide-a (Ph-a); phenoxazine Nile blue derivatives including various phenoxazine dyes; PHOTOFRIN; benzoporphyrin derivative mono-acid; SnET2; and Lutex.

56 Claim 17. The method of Claim 1 wherein said multi-photon photoactivation is a degenerate process.

57 Claim 18. The method of Claim 1 wherein said method for treatment is for photodynamic treatment of disease.

58 Claim 19. The method of Claim 1 wherein said method for treatment is for selective tissue denaturation.

59 Claim 20. The method of Claim 1 wherein said method for treatment is for laser surgery.

60 Claim 21. The method of Claim 1 wherein said method for treatment is for tattoo removal.

61 Claim 22. The method of Claim 1 wherein said multi-photon photoactivation involves  $n$  photons, and wherein  $n$  equals 2 or more photons and can be varied so as to optimize the volume of tissue in which said agent is photoactivated.

62 Claim 23. The method of Claim 1 further comprising the step of controlling the photo-activation by varying the location, irradiance and duration of said light.

63 Claim 24. The method of Claim 3 further comprising the step of varying the pulse energy of said one or more ultrashort pulses to achieve a desired therapeutic process.

64 Claim 25. The method of Claim 24 wherein said pulse energy is set so that said desired therapeutic process is substantially a photophysical process.

65 Claim 26. The method of Claim 24 wherein said pulse energy is set so that said desired therapeutic process is substantially a photochemical process.

66 Claim 27. The method of Claim 1 wherein said multi-photon activation results in electronic excitation of said at least one photo-active agent to a higher quantum mechanically allowed state.

67 Claim 28. The method of Claim 1 wherein said multi-photon activation results in vibrational excitation of said at least one photo-active agent to a higher quantum mechanically allowed state.

68 Claim 29. The method of Claim 1 wherein said multi-photon activation results in photoionization of said at least one photo-active agent.

69 Claim 30. The method of Claim 1 wherein the light to promote said multi-photon excitation of the photo-active agent is an unfocused beam of light.

70 Claim 31. The method of Claim 30 wherein said particular volume of tissue is located substantially at the tissue surface.

71 Claim 32. The method of Claim 30 wherein said particular volume of tissue is located substantially below the tissue surface.

72 Claim 33. The method of Claim 30 further comprising the step of substantially restricting said agent to the particular volume of tissue to be treated.

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claim 1*

Claim 34. A method for producing at least one photo-activated agent in a particular volume of a material, the method comprising treating the particular volume of the material with light to promote a multi-photon excitation of at least one photo-active agent contained in the particular volume of the material, wherein the at least one photo-active agent becomes a photo-activated agent in the particular volume of the material.

Claim 35. The method of Claim 34 wherein the material is pretreated with at least one photo-active agent such that the material retains at least a portion of the at least one photo-active agent at the time that the particular volume of the material is treated with light sufficient to promote said multi-photon excitation of at least one of said at least one photo-active agent.

Claim 36. The method of Claim 34 wherein the material is selected from the group consisting of plant tissue and animal tissue.

Claim 37. The method of Claim 36 wherein said at least one photo-active agent becomes photoactivated in said particular volume at a controllable position.

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Claim 38. The method of Claim 36 wherein said agent is an exogenous agent.

Claim 39. The method of Claim 38 wherein said exogenous agent is selected from

the group comprising psoralen derivatives; porphyrin and hematoporphyrin derivatives; chlorin derivatives; phthalocyanine derivatives; rhodamine derivatives; coumarin derivatives; benzophenoxazine derivatives; chlorpromazine and chlorpromazine derivatives; chlorophyll 5 and bacteriochlorophyll derivatives; pheophorbide a (Pheo a); merocyanine 540 (MC 540); Vitamin D; 5-amino-laevulinic acid (ALA); photosan; pheophorbide-a (Ph-a); phenoxazine Nile blue derivatives including various phenoxazine dyes; PHOTOFRIN; benzoporphyrin derivative mono-acid; SnET2; and Lutex.

Claim 40. The method of Claim 36 wherein said agent is an endogenous agent.

Claim 41. The method of Claim 40 wherein said endogenous agent is selected from the group comprising proteins, natural chromophoric agents including melanin, hemoglobin and carotenes, water, collagen and tattoo dyes.

Claim 42. The method of Claim 34 wherein the light to promote said multi-photon excitation of the photo-active agent is laser light produced by a laser.

Claim 43. The method of Claim 42 wherein the laser light comprises a train of one or more ultrashort pulses.

Claim 44. The method of Claim 43 wherein each of said one or more pulses has a duration of at most approximately 10 ps.

Claim 45. The method of Claim 42 including operating said laser to produce light

at a wavelength between approximately 500 nm to 4000 nm.

Claim 46. The method of Claim 34 wherein said light is at a wavelength between approximately 500 nm to 4000 nm.

Claim 47. The method of Claim 34 wherein the light to promote said multi-photon excitation of the photo-active agent is a focused beam of light.

Claim 48. The method of Claim 47 wherein the focused beam of light is laser light.

Claim 49. The method of Claim 34 wherein said step of treating the particular volume of material includes positioning a focus of a beam of light over a range of positions so that a focal plane of the light beam occurs at a site located between a surface of the material and a point substantially beyond the material surface, whereby said step of treating the particular volume of material may extend to penetrate deep within the material.

Claim 50. The method of Claim 49 wherein said at least one photo-active agent becomes photoactivated in said particular volume at a controllable position substantially beyond a tissue surface.

Claim 51. The method of Claim 34 wherein said treating step includes directing a laser light to said particular volume.

Claim 52. The method of Claim 34 wherein said treating step includes directing a

pulsed laser light to said particular volume.

Claim 53. The method of Claim 52 wherein said laser is pulsed to produce pulses having a duration of at most approximately 10 ps.

Claim 54. The method of Claim 49 further including varying, while the beam of light is extant, the radial position of the focal plane within the material, thereby to photoactivate the at least one photo-active agent at a multiplicity of positions between the material surface and a position located substantially beyond the material surface.

Claim 55. The method of Claim 34 wherein said multi-photon photoactivation includes an essentially simultaneous interaction of at least two photons with said agent so as to produce a photo-activated agent.

Claim 56. The method of Claim 34 wherein said multi-photon photoactivation is a degenerate process.

Claim 57. The method of Claim 36 wherein said method for treatment is for photodynamic treatment of disease.

Claim 58. The method of Claim 36 wherein said method for treatment is for selective tissue denaturation.

Claim 59. The method of Claim 36 wherein said method for treatment is for laser

surgery.

Claim 60. The method of Claim 36 wherein said method for treatment is for tattoo removal.

Claim 61. The method of Claim 34 wherein said multi-photon photoactivation involves  $n$  photons, and wherein  $n$  equals 2 or more photons and can be varied so as to optimize the volume of material in which said agent is photoactivated.

Claim 62. The method of Claim 34 further comprising the step of controlling the photo-activation by varying the location, irradiance and duration of said light.

Claim 63. The method of Claim 43 further comprising the step of varying the pulse energy of said one or more ultrashort pulses to achieve a desired therapeutic process.

Claim 64. The method of Claim 63 wherein said pulse energy is set so that said desired therapeutic process is substantially a photophysical process.

Claim 65. The method of Claim 63 wherein said pulse energy is set so that said desired therapeutic process is substantially a photochemical process.

Claim 66. The method of Claim 34 wherein said multi-photon activation results in electronic excitation of said at least one photo-active agent to a higher quantum mechanically allowed state.

Claim 67. The method of Claim 34 wherein said multi-photon activation results in vibrational excitation of said at least one photo-active agent to a higher quantum mechanically allowed state.

Claim 68. The method of Claim 34 wherein said multi-photon activation results in photoionization of said at least one photo-active agent.

Claim 69. The method of Claim 34 wherein the light to promote said multi-photon excitation of the photo-active agent is an unfocused beam of light.

Claim 70. The method of Claim 69 wherein said particular volume of material is located substantially at a surface of the material.

Claim 71. The method of Claim 69 wherein said particular volume of tissue is located substantially below a surface of the material.

Claim 72. The method of Claim 69 further comprising the step of substantially restricting said agent to the particular volume of material tissue to be treated.

Claim 73. A method for the medical treatment of a particular volume of tissue wherein the tissue includes at least one photo-active agent, the method comprising the steps of:

5      directing light to specific regions of interest within the tissue, including regions substantially below a tissue surface, said light being selected to penetrate the tissue and to

promote multi-photon excitation substantially only at a focal zone;

controlling the location of said focal zone over a range of depths within said tissue;

and

using multi-photon excitation, photoactivating at least one of said at least one agent

10 over said range of depths within said tissue, thereby producing at least one photo-activated agent substantially only at the focal zone.

Claim 74. The method of Claim 73 wherein said directing step includes directing a laser light produced by a laser to said particular volume.

Claim 75. The method of Claim 73 wherein said directing step includes directing one or more ultrashort laser pulses to said particular volume.

Claim 76. The method of Claim 75 wherein said laser is operated to produce pulses, each of said pulses having a duration of at most approximately 10 ps.

Claim 77. The method of Claim 73 wherein the tissue is pretreated with at least one photo-active agent such that the tissue retains at least a portion of said at least one photo-active agent at the time that the particular volume of the tissue is treated with light sufficient to promote a multi-photon excitation of at least one of said at least one photo-active agent.

Claim 78. The method of Claim 73 wherein said agent is an endogenous agent.

Claim 79. The method of Claim 78 wherein said endogenous agent is selected from the group comprising proteins, natural chromophoric agents including melanin, hemoglobin and carotenes, water, collagen and tattoo dyes.

Claim 80. The method of Claim 73 wherein said agent is an exogenous agent.

Claim 81. The method of Claim 80 wherein said exogenous agent is selected from the group comprising psoralen derivatives; porphyrin and hematoporphyrin derivatives; chlorin derivatives; phthalocyanine derivatives; rhodamine derivatives; coumarin derivatives; benzophenoxazine derivatives; chlorpromazine and chlorpromazine derivatives; chlorophyll and bacteriochlorophyll derivatives; pheophorbide a (Pheo a); merocyanine 540 (MC 540); Vitamin D; 5-amino-laevulinic acid (ALA); photosan; pheophorbide-a (Ph-a); phenoxazine Nile blue derivatives including various phenoxazine dyes; PHOTOFRIN; benzoporphyrin derivative mono-acid; SnET2; and Lutex.

Claim 82. The method of Claim 73 wherein the light to promote said multi-photon excitation of the photo-active agent is laser light having a wavelength from approximately 500 nm to 4000 nm.

Claim 83. The method of Claim 73 wherein the light to promote said multi-photon excitation of the photo-active agent is a focused beam of light.

Claim 84. The method of Claim 83 wherein the focused beam of light is laser light.

Claim 85. The method of Claim 73 wherein the light to promote said multi-photon excitation of the photo-active agent is an unfocused beam of light.

Claim 86. The method of Claim 85 wherein said regions of interest are located substantially at the tissue surface.

Claim 87. The method of Claim 85 wherein said regions of interest are located substantially below the tissue surface.

Claim 88. The method of Claim 85 further comprising the step of substantially restricting said agent to the particular volume of tissue to be treated.

Claim 89. The method of Claim 73 wherein said step of treating the particular volume of the plant or animal tissue includes positioning a focus of a beam of light over a range of positions so that a focal plane of the light beam occurs at a site located between a surface of the tissue and a point substantially beyond the tissue surface, whereby said step 5 of treating the particular volume of the plant or animal tissue may extend to penetrate deep within the tissue.

Claim 90. The method of Claim 73 wherein said at least one photo-active agent becomes photoactivated in said particular volume at a controllable position substantially beyond a tissue surface.

Claim 91. The method of Claim 73 further including varying, while the beam of light is extant, the radial position of the focal plane within the tissue, thereby to photoactivate the at least one photo-active agent at a multiplicity of positions between the tissue surface and a position located substantially beyond the tissue surface.

Claim 92. The method of Claim 73 wherein said multi-photon photoactivation includes an essentially simultaneous interaction of at least two photons with said agent so as to produce a photo-activated agent.

Claim 93. The method of Claim 73 wherein said multi-photon photoactivation is a degenerate process.

Claim 94. The method of Claim 73 wherein said method for treatment is for photodynamic treatment of disease.

Claim 95. The method of Claim 73 wherein said method for treatment is for selective tissue denaturation.

Claim 96. The method of Claim 73 wherein said method for treatment is for laser surgery.

Claim 97. The method of Claim 73 wherein said method for treatment is for tattoo removal.

Claim 98. The method of Claim 73 wherein said multi-photon photoactivation involves n photons, and wherein n equals 2 or more photons and can be varied so as to optimize the volume of tissue in which said agent is photoactivated.

Claim 99. The method of Claim 73 further comprising the step of controlling the photo-activation by varying the location, irradiance and duration of said light.

Claim 100. The method of Claim 75 further comprising the step of varying the pulse energy of said one or more ultrashort pulses to achieve a desired therapeutic process.

Claim 101. The method of Claim 100 wherein said pulse energy is set so that said desired therapeutic process is substantially a photophysical process.

Claim 102. The method of Claim 100 wherein said pulse energy is set so that said desired therapeutic process is substantially a photochemical process.

Claim 103. The method of Claim 73 wherein said multi-photon activation results in electronic excitation of said at least one photo-active agent to a higher quantum mechanically allowed state.

Claim 104. The method of Claim 73 wherein said multi-photon activation results in vibrational excitation of said at least one photo-active agent to a higher quantum mechanically allowed state.

Claim 105. The method of Claim 73 wherein said multi-photon activation results in photoionization of said at least one photo-active agent.

Claim 106. The method of Claim 73 wherein said photoactivating step includes using energy of a first photon to excite at least one of said at least one agent to a transient virtual level between an initial state and an excited state and using energy of at least a second photon to excite said agent to a quantum mechanically allowed excited state before said agent makes a transition back to the initial state.

Claim 107. A method for the treatment of a particular volume of plant or animal tissue, the tissue including at least one photoactive agent in the particular volume, the method comprising:

illuminating said particular volume of tissue to cause multi-photon excitation of at  
5 least one of said at least one photo-active agent,

wherein said at least one photo-active agent at a site of the multi-photon excitation is firstly excited to a transient virtual state and secondly excited to a quantum mechanically allowed excited state and wherein the at least one excited photo-active agent becomes photo-activated in the particular volume.

Claim 108. The method of Claim 107 including the treatment of a particular volume of plant or animal tissue located substantially below a tissue surface.

Claim 109. The method of Claim 108 wherein said illumination with light and said transient virtual state occurs substantially only at said particular volume, despite the passage

of light through other tissue portions between said surface and said particular volume.

Claim 110. The method of Claim 109 further including varying the position where multi-photon excitation occurs over a range of depths below the tissue surface.

Claim 111. The method of Claim 107 wherein said illuminating step includes directing a laser beam produced by a laser to said particular volume.

Claim 112. The method of Claim 107 wherein said illuminating step includes directing an ultrashort pulsed laser beam having one or more pulses of a duration of at most approximately 10 ps to said particular volume.

Claim 113. The method of Claim 112 wherein an individual photon provided by said pulsed laser beam has insufficient energy to directly excite the agent from a ground state to an excited electronic state.

Claim 114. The method of Claim 107 wherein said multi-photon photoactivation is a degenerate process.

Claim 115. The method of Claim 107 wherein said multi-photon photoactivation involves  $n$  photons, and wherein  $n$  equals 2 or more photons and can be varied so as to optimize the volume of tissue in which said agent is photoactivated.

Claim 116. The method of Claim 107 including treatment of a particular volume of

plant or animal tissue located substantially at a tissue surface.

~~Claim 117. Apparatus for treating a particular volume of plant or animal tissue containing at least one photo-active agent, the apparatus comprising:~~

~~a source of light, said light having a frequency effective to penetrate substantially into the tissue, said light being adapted to promote multi-photon excitation of the agent 5 contained within the tissue; and~~

~~focusing apparatus for focusing the light throughout a range of focal lengths extending from a surface of said tissue to a depth substantially beyond said surface, said light source and focusing apparatus cooperating to promote multi-photon excitation of the agent; wherein a focal point or focal plane is adjustable with respect to said light source.~~

Claim 118. The apparatus of Claim 117 wherein the light to promote said multi-photon photoactivation is a laser light produced by a laser.

Claim 119. The apparatus of Claim 118 wherein the laser light comprises one or more ultrashort pulses.

Claim 120. The apparatus of Claim 119 wherein each of said one or more pulses has a duration of at most approximately 10 ps.

Claim 121. The apparatus of Claim 118 including operating the laser to produce light at a wavelength between approximately 500 nm to 4000 nm.

Claim 122. The apparatus of Claim 117 wherein said light is at a wavelength from approximately 500 nm to 4000 nm.

Claim 123. The apparatus of Claim 117 wherein said agent is an exogenous agent.

Claim 124. The apparatus of Claim 123 wherein said agent is selected from the group comprising psoralen derivatives; porphyrin and hematoporphyrin derivatives; chlorin derivatives; phthalocyanine derivatives; rhodamine derivatives; coumarin derivatives; benzophenoxazine derivatives; chlorpromazine and chlorpromazine its derivatives; chlorophyll 5 and bacteriochlorophyll derivatives; pheophorbide a (Pheo a); merocyanine 540 (MC 540); Vitamin D; 5-amino-laevulinic acid (ALA); photosan; pheophorbide-a (Ph-a); phenoxazine Nile blue derivatives including various phenoxazine dyes; PHOTOFRIN; benzoporphyrin derivative mono-acid; SnET2; and Lutex.

Claim 125. The apparatus of Claim 117 wherein said agent is an endogenous agent.

Claim 126. The apparatus of Claim 125 wherein said endogenous agent is selected from the group comprising proteins, natural chromophoric agents including melanin, hemoglobin and carotenes, water, collagen and tattoo dyes.

Claim 127. Apparatus for administering a photodynamic medical treatment comprising:

light source means for directing a confined light at and into tissue to be treated, said light being selected in frequency and energy to penetrate below a tissue surface and to

5 promote multi-photon excitation substantially only at a focal zone; and  
means for varying a position of a focal region of the light within a range of depths  
in the tissue to be treated so that an agent within the tissue becomes photoactivated using  
multi-photon excitation.

Claim 128. The apparatus of Claim 127:

wherein the light source means includes means for producing a collimated light beam;  
and

wherein the light source means includes focusing means for focusing the collimated  
light beam to a focal zone located with tissue at a point below the tissue surface.

Claim 129. Apparatus for administering a photodynamic medical treatment  
comprising:

light source means for directing unfocused light at and into tissue to be treated, said  
light being selected in frequency and energy to promote multi-photon excitation so that an  
agent within the tissue becomes photoactivated using multi-photon excitation.

Claim 130. The apparatus of Claim 129 wherein a treatment zone is located  
substantially at a surface of the tissue.

Claim 131. The apparatus of Claim 129 wherein a treatment zone is located  
substantially below a surface of the tissue.

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Claim 132. The method of Claim 1 further comprising the step of selecting a

wavelength for said light to promote said multi-photon activation so as to optimize the efficiency and selectivity of photo-activation of said at least one photoactive agent within said volume of tissue.

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Claim 133. The method of Claim 34 further comprising the step of selecting a wavelength for said light to promote said multi-photon excitation so as to optimize the efficiency and selectivity of photo-activation of said at least one photoactive agent within said volume of material.

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Claim 134. The method of Claim 73 further comprising the step of selecting a wavelength for said light to promote said multi-photon activation so as to optimize the efficiency and selectivity of photo-activation of said at least one photoactive agent within said volume of tissue.

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Claim 135. The method of Claim 109 further comprising the step of selecting a wavelength for said light to promote said multi-photon activation so as to optimize the efficiency and selectivity of photo-activation of said at least one photoactive agent within said volume of tissue.

Claim 136. The apparatus of Claim 117 wherein a wavelength for said light adapted to promote multi-photon excitation is selected so as to optimize the efficiency and selectivity of photo-activation of said agent.

Claim 137. The apparatus of Claim 127 wherein a wavelength for said light adapted

to promote multi-photon excitation is selected so as to optimize the efficiency and selectivity of photo-activation of said agent.

Claim 138. The apparatus of Claim 129 wherein a wavelength for said light adapted to promote multi-photon excitation is selected so as to optimize the efficiency and selectivity of photo-activation of said agent.